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THE EFFECTS OF HYPOGLYCAEMIA ON CEREBRAL BLOOD FLOW AND METABOLISM IN THE NEW-BORN CALF

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SUMMARY

1. The effects of insulin hypoglycaemia on cerebral blood flow and metabolism have been examined in unanaesthetized, unrestrained calves between 1 and 26 days after birth.

2. Cerebral blood flow was measured with an inert gas technique using molecular hydrogen, and cerebral metabolism was quantified by determination of arterio-cerebral venous ($A - V$) concentration differences for oxygen, glucose, lactate, pyruvate, acetoacetate, β-D-hydroxybutyrate and ammonia.

3. During normoglycaemia the mean ($A - V$) difference for glucose was close to one sixth that of oxygen, on a molar basis. A small net loss of pyruvate from the brain was found, but there was no significant ($A - V$) difference for lactate. Arterial concentrations of acetoacetate and β-D-hydroxybutyrate were low, and no utilization of ketone bodies by the brain was demonstrated.

4. Moderate hypoglycaemia (arterial plasma glucose concentration 1–2 m-mole/l.) had no measurable effect on either cerebral blood flow or metabolism.

5. During profound hypoglycaemia (arterial plasma glucose concentration $< 1{\cdot}0$ m-mole/l.) cerebral glucose uptake was sufficient to account for only 56% of the cerebral oxygen consumption. Cerebral oxygen consumption fell in comatose animals, but increased during hypoglycaemic convulsions, as did cerebral blood flow.

6. In day-old calves hypoglycaemia was associated with a rise in blood lactate concentration and uptake of lactate by the brain.

7. A net loss of ammonia by the brain was observed during hypoglycaemia in calves at all ages examined. The loss was greater in convulsing than in comatose animals.

INTRODUCTION

Although measurement of cerebral blood flow and metabolism *in vivo* has been possible since the introduction of a valid, quantitative method for determination of cerebral blood flow by Kety & Schmidt (1945), there have been few studies in the new-born of any mammalian species. Quantitative procedures are too invasive for application in the new-born infant, and animal preparations have either required anaesthesia (Purves & James, 1969; Hernandez, Brennan, Vannucci & Bowman, 1978; Gregoire, Gjedde, Plum & Duffy, 1978) or not allowed the simultaneous measurement of both flow and metabolism (Barker, 1965; Douglas Jones, Burd, Makowski, Meschia & Battaglia, 1975).

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The effects of insulin-induced hypoglycaemia on cerebral metabolism have been investigated extensively, both by studying the exchange of metabolites between the brain and circulation (Dameshek & Myerson, 1935; Himwich & Fazekas, 1937; Kety, Woodford, Harmel, Freyhan, Appel & Schmidt, 1948; Pappenheimer & Setchell, 1973; Norberg & Siesjö, 1976, and by direct biochemical estimations on brain tissue (Tews, Carter & Stone, 1965; Ferrendelli & Chang, 1973; Lewis, Ljunggren, Norberg & Siesjö, 1974). Its effect on cerebral blood flow has been investigated in man (Kety *et al.* 1948; Della Porta, Maolo, Negri & Rossella, 1964; Eisenberg & Seltzer, 1962) and in animals under anaesthesia (Norberg & Siesjö, 1976). There is little information however concerning the effects of hypoglycaemia on cerebral blood flow and metabolism in the mammalian new-born, although a number of observations suggest that these might be different in the neonatal period. Both the infant and the calf for example are known to be exceptionally tolerant of low blood glucose concentrations during the period immediately after birth (Shelley & Neligan, 1966; Edwards, 1964).

The experiments described in the present paper were undertaken to establish the normal values for cerebral blood flow and oxidative metabolism in the new-born calf, and to determine the changes which occur during insulin-induced hypoglycaemia. Cerebral blood flow was measured using a modification of the inert gas method of Kety & Schmidt (1945), (Gardiner, 1978), which allowed measurements to be made in unrestrained, unanaesthetized animals.

METHODS

Animals

Pedigree Jersey calves were obtained from local farms shortly after birth and used at ages ranging from 1 to 26 days (20–34 kg body weight). Animals used on the second day after birth were unsuckled. Other animals were kept in individual pens in the laboratory animal house and maintained on a diet of milk (6–8 pints/day). Food was withheld for at least 14 hr prior to surgery. Daily records were kept of the weight and rectal temperature of each animal and care was taken to avoid using animals that were not completely healthy.

Surgical procedure

Anaesthesia was induced with chloroform and maintained with halothane (Fluothane; I.C.I. Ltd.) in oxygen, administered by means of a standard closed circuit system attached to an endotracheal tube.

Narrow-bore polyethylene catheters were inserted into the saphenous artery and vein on one side so that their tips lay in the abdominal aorta and interior vena cavá respectively. The arterial catheter was used later to monitor aortic blood pressure and heart rate and for collection of arterial blood samples. A catheter-mounted platinum electrode was inserted into the saphenous artery on the other side so that its tip lay in the abdominal aorta. Sintered silver/silver chloride reference electrodes (Tektronix, ECG) were positioned subcutaneously on the back anterior to the iliac crest, and at the base of the neck. A short polyethylene cannula for administration of hydrogen gas was sutured into position so that its tip lay well inside the nasal airway.

With the animal's head in a supportive frame, a cruciate incision was made just posterior to the frontal eminence, and the scalp and underlying periosteum retracted. A trephine hole 1·5 cm in diameter was made in the parietal bone in the mid line immediately behind the junction of sagittal and coronal sutures. A needle was used to make two holes in the roof of the sagittal dural sinus through which were inserted a narrow bore polyethylene catheter for blood sampling, and a catheter-mounted platinum electrode. The trephine hole was partly closed by a perforated Perspex plug, and the residual spaces filled with plaster of paris.

Measurement of cerebral blood flow

Cerebral blood flow was measured using a modification of the inert gas technique of Kety & Schmidt (1945) based on the indirect Fick principle. Molecular hydrogen gas was used as the indicator (Aukland, Bower & Berliner, 1964; Gotoh, Meyer & Tomita, 1966), and the desaturation curves for flow calculation (McHenry, 1963). The partial pressure of hydrogen (P_{H_2}) was measured by means of platinum electrodes implanted intravascularly which allowed measurements to be made in unanaesthetized, unrestrained animals. The method has been demonstrated to the Physiological Society (Gardiner, 1978).

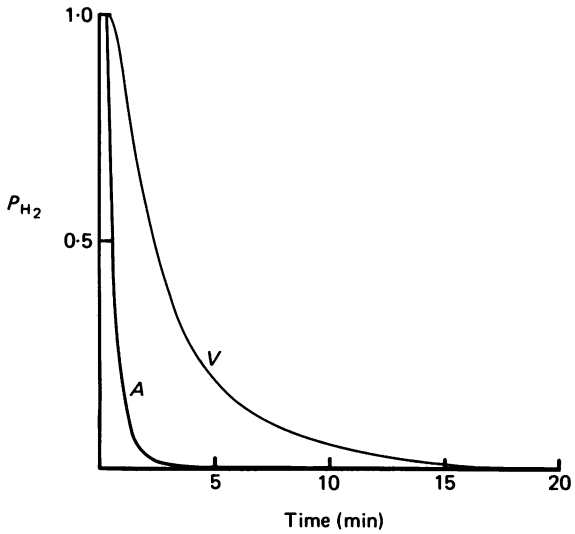

Fig. 1. Arterial (A) and cerebral venous (V) hydrogen clearance curves. P_{H_2} arbitrary scale. Cerebral blood flow = 34 ml. 100 g^{-1}. min^{-1}.

The electrodes consisted of 250 μm diameter platinum wire sealed in soda glass and mounted in a polyethylene catheter. The exposed end was platinized and coated with a polystyrene membrane. The electrodes were maintained at a potential of +700 mV with respect to the silver/silver chloride reference electrode. An optical coupler was used to connect the electrode signal to mains-driven recording equipment. Close matching of electrode characteristics *in vivo* was demonstrated by simultaneous recording of P_{H_2} desaturation curves from pairs of electrodes placed in the sagittal dural sinus. The mean difference in the area beneath the curve recorded with eight pairs of electrodes was $7 \pm 2\%$.

For each flow measurement hydrogen gas was added to the animal's inspired air via the nasal cannula at a constant and reproducible flow rate, to achieve and maintain an alveolar hydrogen concentration of about 5%. Administration of hydrogen was continued until the cerebral venous P_{H_2} curve reached a plateau, at which time it may be assumed that saturation of brain tissue has occurred, and that the arterial and cerebral venous P_{H_2} are equal (Gotoh *et al.* 1966). The gain of each electrode was then adjusted to compensate for any differences in electrode sensitivity, administration of hydrogen discontinued, and cerebral blood flow calculated from the ensuing desaturation curves (Fig. 1) using the Kety-Schmidt formula. A blood-brain partition coefficient for hydrogen of 1·0 (Fieschi, Bozzao & Agnoli, 1965) was assumed, and t taken as the time at which cerebral venous P_{H_2} became indistinguishable from zero.

A steady state for flow during each determination was inferred from the constancy of the arterio – cerebral venous oxygen content differences measured at the beginning and end of desaturation. It is not technically feasible to exclude completely the possibility that some contamination of sagittal sinus blood with extra-cerebral blood may occur via the ethmoidal

veins or diploic anastamoses. However, the mono-exponential nature of the hydrogen desaturation curves recorded from the sagittal sinus provides indirect evidence that any such contamination is small, as a significant low-flow compartment in the drainage field gives rise to a prolonged tail (Nilsson & Siesjö, 1976) (Fig. 2).

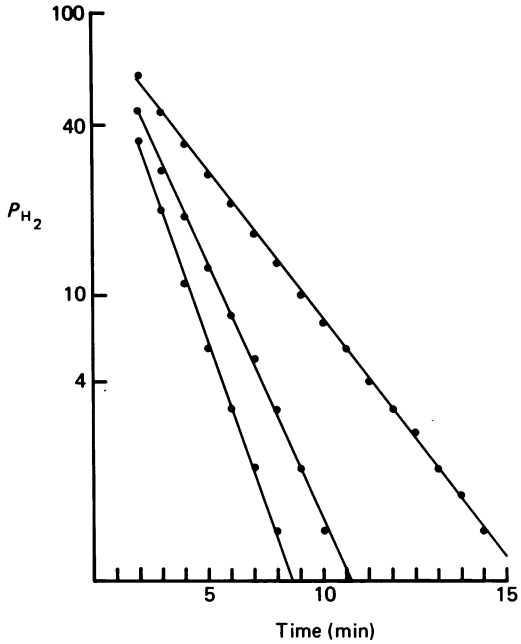

Fig. 2. Semi-logarithmic plots of cerebral venous hydrogen clearance curves. The initial 2 min is distorted by arterial reçirculation and has been omitted.

Experimental procedure

Measurements were made in unanaesthetized, unrestrained animals after full recovery from surgery. The majority of animals were standing quietly and able to feed within 2 hr of the end of the operation. Heart rate and aortic blood pressure were monitored continuously using a Devices L221 pressure transducer connected to a Devices M2 recorder. Samples for metabolite estimations were withdrawn simultaneously from the aorta and the sagittal dural sinus at the beginning and end of each flow determination. Observations were made during normoglycaemia and at various times following the intravenous administration of insulin (Soluble Insulin B.P., Wellcome, 4 i.u./kg body wt.).

Analytical procedure

Arterial and cerebral venous blood samples were collected into heparinized tubes containing sodium fluoride and centrifuged immediately at +4 °C. Plasma glucose was estimated in triplicate with glucose oxidase by means of a Beckman Glucose Analyser Mark 2. Samples for ammonia estimation were collected into tubes containing Ca EDTA and centrifuged immediately at +4 °C. Plasma ammonia was estimated in duplicate, within 2 hr of sample collection, using a specific enzymic technique (Da Fonseca-Wollheim, 1973).

Blood samples for metabolite estimations were deproteinized immediately in equal volumes of ice-cold perchloric acid (10% w/v). Pyruvate, acetoacetate and β-D-hydroxybutyrate were determined enzymically with minor modifications of the methods described in Bergmeyer (1974), and lactate as described by Barker & Britton (1957). Ketones were assayed within 24 hr of collection.

Samples for blood gas and pH estimations were collected anaerobically into syringes the tips of which were filled with heparin in saline (10 i.u./ml.). Oxygen contents were measured in duplicate using a polarographic method described by Linden, Ledsome & Norman (1965), and blood gas tensions and pH measured by means of a Radiometer PHM 72.

RESULTS

A – V differences across the brain

The mean arterial concentration and arterio – cerebral venous concentration differences for a number of metabolites during normoglycaemia and hypoglycaemia are given in Table 1.

A – V differences (AVD) in blood glucose concentration were calculated from values measured on plasma, and the packed cell volume (PCV), using the formula: blood AVD = plasma AVD × (1-PCV). In thirty-five pairs of arterial and sagittal-sinus samples collected simultaneously, the directly measured blood glucose AVD was $0{\cdot}41 \pm 0{\cdot}03$ m-mole/l. and that calculated from the plasma glucose AVD was $0{\cdot}38 \pm 0{\cdot}04$ m-mole/l.

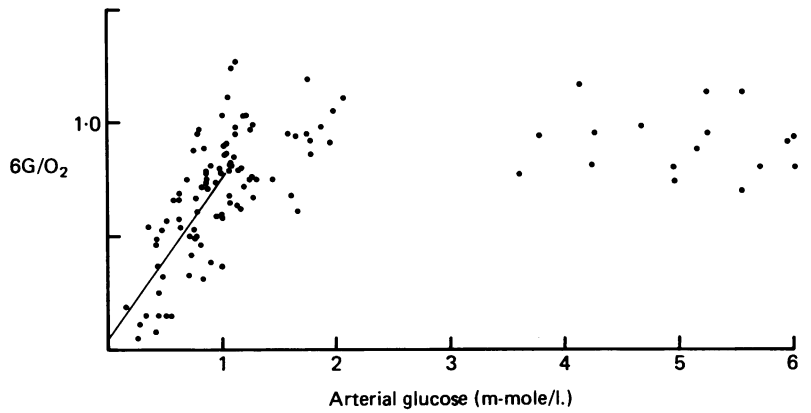

Fig. 3. Glucose–Oxygen Index, $6\ G/O_2$, i.e.

$$\frac{(6 \times \text{AVD glucose}) - (\text{AVD pyruvate}/2)}{\text{AVD oxygen}}$$

at different arterial plasma glucose concentrations. The continuous line is the regression line for values at glucose concentrations < 1·0 m-mole/l. ($r = 0{\cdot}66$).

During normolgycaemia mean glucose uptake was found to be just less than that required to account for the observed cerebral oxygen consumption (Glucose–Oxygen Index $6\ G/O_2 = 0{\cdot}90 \pm 0{\cdot}03$). No significant AVD for lactate was found, but there was a statistically significant ($P < 0{\cdot}001$) though quantitatively trivial negative AVD for pyruvate. Arterial concentrations of acetoacetate and β-D-hydroxybutyrate were low, and no significant $A - V$ differences for these metabolites were observed during either normoglycaemia or hypoglycaemia.

During moderate hypoglycaemia the small negative AVD for pyruvate disappeared, but there was no measurable change in the near stoichiometric relationship between glucose and oxygen uptake. During profound hypoglycaemia this stoichiometric relationship was lost, and glucose uptake was only sufficient to account for $56 \pm 3\%$ of the observed cerebral oxygen consumption. Individual values for the Glucose–Oxygen Index are plotted against arterial plasma glucose concentration in Fig. 3 ($r = 0{\cdot}66$ at arterial plasma glucose concentrations below 1 m-mole/l.).

TABLE 1. Arterio–cerebral venous concentration differences (AVD) during normolgycaemia and hypoglycaemia in the calf. Mean age 8 ± 1 days. Values are mean ± S.E. of mean. Art.: arterial concentration. Packed cell volume (PCV) = 36 ± 2%. Blood glucose AVD = plasma AVD × (1-PCV)

$$6\ G/O_2 = \frac{((6 \times \text{AVD glucose}) - (\text{AVD pyruvate}/2))}{\text{AVD oxygen}}$$

	Plasma glucose (m-mole/l.)		Blood glucose (m-mole/l.)	Oxygen (m-mole/l.)		Pyruvate (m-mole/l.)	
	Art.	AVD	AVD	Art.	AVD	Art.	AVD
Normoglycaemia	5·25 ± 0·28	0·52 ± 0·04	0·33 ± 0·02	5·39 ± 0·31	2·20 ± 0·13	0·10 ± 0·02	−0·02 ± 0·00
Hypoglycaemia							
Moderate	1·31 ± 0·09	0·44 ± 0·03	0·28 ± 0·01	4·90 ± 0·17	1·85 ± 0·09	0·12 ± 0·02	0·00 —
Profound	0·68 ± 0·03	0·23 ± 0·03	0·15 ± 0·01	5·34 ± 0·33	1·61 ± 0·09	0·17 ± 0·02	0·00 —

	Acetoacetate (m-mole/l.)		β-D-hydroxybutyrate (m-mole/l.)		
	Art.	AVD	Art.	AVD	$6\ G/O_2$
Normoglycaemia	0·027 ± 0·006	0·004 ± 0·003	0·13 ± 0·03	0·01 ± 0·01	0·90 ± 0·03
Hypolgycaemia					
Moderate	— —	— —	— —	— —	0·91 ± 0·03
Profound	0·026 ± 0·006	0·001 ± 0·004	0·08 ± 0·02	0·00 —	0·56 ± 0·03

TABLE 2. Cerebral blood flow, glucose utilization and oxygen consumption during normoglycaemia and hypoglycaemia in the calf. Values are mean ± S.E. of mean. CBF: cerebral blood flow. CMR: cerebral metabolic rate. MABP: mean arterial blood pressure

	n	Arterial plasma glucose conc. (m-mole/l.)	MABP (mmHg)	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH	CBF (ml. 100 g^{-1}. min^{-1})	$CMR_{glucose}$ (μmole. 100 g^{-1}. min^{-1})	CMR_{oxygen} (μmole. 100 g^{-1}. min^{-1})
Normoglycaemia	20	5·25	97	66	41	7·43	44	15	94
		± 0·28	± 4	± 2	± 1	—	± 2	± 1	± 4
Hypoglycaemia									
Moderate	23	1·31	82	61	40	7·41	45	13	88
		± 0·07	± 2	± 2	± 1	—	± 3	± 1	± 6
Profound									
Group I	20	0·74	84	63	41	7·39	47	6	71
		± 0·05	± 2	± 2	± 1	—	± 5	± 1	± 7
Group II	5	0·56	87	65	36	7·42	125	12	143
		± 0·12	± 9	± 5	± 2	—	± 18	± 4	± 12

TABLE 3. Cerebral lactate uptake during hypoglycaemia in day-old calves. n = 11. Values are mean ± S.E. of mean. Art.: arterial concentration. AVD: arterio – cerebral venous concentration difference. CMR: cerebral metabolic rate. CBF: cerebral blood flow

Plasma glucose	Lactate		CMR_{oxygen}	$CMR_{glucose}$	$CMR_{lactate}$	CBF
Art. (m-mole/l.)	Art. (m-mole/l.)	AVD (m-mole/l.)	(μmole. 100 g^{-1}. min^{-1})	(μmole. 100 g^{-1}. min^{-1})	(μmole. 100 g^{-1}. min^{-1})	(ml. 100 g^{-1}. min^{-1})
0·69	3·08	0·10	85	6·2	5·9	48
	± 0·30	± 0·03	± 9	± 1·2	± 1·6	—

Cerebral blood flow, glucose utilization and oxygen consumption

Values for cerebral blood flow, glucose utilization and oxygen consumption during normoglycaemia and hypoglycaemia, together with corresponding values for a number of relevant physiological variables are shown in Table 2.

Mean arterial blood pressure, arterial blood gas tensions and packed cell volume, all of which may affect blood flow through the brain, were within the normal range. During normolgycaemia mean cerebral blood flow was 44 ± 2 ml. $100\ g^{-1}.min^{-1}$, mean cerebral oxygen consumption was 94 ± 4 μmole. $100\ g^{-1}.min^{-1}$ and mean cerebral glucose uptake 15 ± 1 μmole. $100\ g^{-1}.min^{-1}$. A small net release of pyruvate from the brain, 0·79 μmole. $100\ g^{-1}.min^{-1}$, was observed, equivalent to less than 3 % of the glucose uptake.

The changes which occurred during hypoglycaemia varied with the degree of hypoglycaemia and the behavioural state of the animals. During moderate hypoglycaemia (mean arterial plasma glucose concentration $1{\cdot}31 \pm 0{\cdot}09$ m-mole/l.) most animals showed no abnormal signs other than transient initial hyperventilation and no significant changes were observed in either cerebral blood flow or metabolism.

Observations made during profound hypoglycaemia (mean arterial plasma glucose concentration < 1·0 m-mole/l.) have been divided into two groups according to the behavioural state of the animal. Group I animals, designated 'comatose', showed signs of somnolence and lethargy but remained conscious and responsive to sensory stimuli such as noise. Group II animals, designated 'convulsing', were exhibiting clonic generalized seizures together with nystagmus.

In the animals which became 'comatose' mean cerebral glucose consumption fell from 15 ± 1 to 6 ± 1 μmole. $100\ g^{-1}.min^{-1}$ ($P < 0{\cdot}001$), while mean cerebral oxygen consumption fell from 94 ± 4 to 71 ± 7 μmole. $100\ g^{-1}.min^{-1}$ ($0{\cdot}01 > P > 0{\cdot}001$), but there was no significant change in cerebral blood flow. In 'convulsing' animals however, there was a significant rise in both cerebral blood flow (to 125 ± 18 ml. $100\ g^{-1}.min^{-1}$; $P < 0{\cdot}001$), and oxygen consumption (to 143 ± 12 μmole. $100\ g^{-1}.min^{-1}$; $P < 0{\cdot}001$). Mean cerebral glucose uptake was higher than that in 'comatose' hypoglycaemic animals, but still only sufficed to account for about half the cerebral oxygen consumption.

Lactate metabolism

Hypoglycaemia was associated with a rise in blood lactate concentration which was more marked in 24-hr-old animals. At this age mean arterial lactate concentration rose from $1{\cdot}96 \pm 0{\cdot}10$ to $3{\cdot}08 \pm 0{\cdot}30$ m-mole/l. during profound hypoglycaemia ($n = 6$). A smaller rise in mean arterial lactate concentration occurred in six older calves; from $1{\cdot}51 \pm 0{\cdot}10$ to $2{\cdot}12 \pm 0{\cdot}20$ m-mole/l. In the day-old calves there was a mean net uptake of lactate by the brain of $5{\cdot}9 \pm 1{\cdot}6$ μmole. $100\ g^{-1}.min^{-1}$ during hypoglycaemia (equivalent, if oxidized, to 21 % of the observed cerebral oxygen consumption (Table 3)), whereas no net uptake of lactate by the brain was observed in older animals at any stage.

Ammonia

The mean arterial plasma ammonia concentration in a group of fifteen calves aged between 1 and 22 days was $42 \pm 0{\cdot}40$ μmole/l. No $(A - V)$ difference for ammonia

was detectable during normoglycaemia, and no significant change in arterial plasma ammonia concentration occurred during hypoglycaemia.

In eighteen measurements made during hypoglycaemia at a mean arterial plasma glucose concentration of $1{\cdot}06 \pm 0{\cdot}10$ m-mole/l., when the animals were showing signs of somnolence and lethargy, a significant negative $(A - V)$ difference for ammonia was found of 8 ± 2 μmole/l. corresponding to a net loss of ammonia from the brain of $0{\cdot}25 \pm 0{\cdot}06$ μmole.100 g^{-1}.min^{-1}. Seven measurements made during convulsions, at a mean arterial plasma glucose concentration of $0{\cdot}9 \pm 9{\cdot}2$ m-mole/l., revealed that there was a significant negative $(A - V)$ difference under these conditions of 18 ± 5 μmole/l. corresponding to a significantly higher rate of ammonia loss ($0{\cdot}98 \pm 0{\cdot}16$ μmole.100 g^{-1}.min^{-1} ($P < 0{\cdot}001$).

On four occasions measurements were repeated at least 30 min after the start of continuous intravenous infusions of glucose, sufficient to raise the mean arterial plasma glucose concentration from $0{\cdot}88 \pm 0{\cdot}09$ to $2{\cdot}67 \pm 0{\cdot}47$ m-mole/l. This was associated with a significant fall in the rate at which ammonia was released from the brain, from $0{\cdot}65 \pm 0{\cdot}10$ to $0{\cdot}09 \pm 0{\cdot}05$ μmole.100 g^{-1}.min^{-1}.

DISCUSSION

The simultaneous determination of arterio–cerebral venous concentration differences and cerebral blood flow has formed the basis of most investigations of the effects of hypoglycaemia on cerebral metabolism *in vivo*. This approach has the advantage that measurements can be made on the intact brain, but the information it provides is limited in several important respects. Changes in local flow and metabolism are not revealed, and no data emerges concerning those substances which are formed and consumed entirely within the brain or undergo no net exchange between the brain and blood, such as energy rich phosphate molecules or neurotransmitters. In addition, the metabolic fate or origin within the brain of those substances for which net exchange with the circulation is measurable can only be inferred. Nevertheless, it represents the only means by which the effects of hypoglycaemia on over-all cerebral blood flow and metabolism can be determined *in vivo* and has not previously been applied to the new-born animal for this purpose.

The results show that under normal conditions the pattern of over-all cerebral oxidative metabolism in the new-born calf is not qualitatively different from that which has long been accepted as normal in the mature mammalian brain. The measured glucose uptake was very nearly sufficient to account, if fully oxidized, for the observed cerebral oxygen consumption. There was no evidence for a substantial anaerobic component of cerebral glucose metabolism, or for utilization of substrates other than glucose. This is in accord with evidence obtained by comparison of the molar ratios of $A - V$ differences across the brain for oxygen, glucose, lactate and pyruvate in the new-born infant (Kraus, Schlenker & Schwedesky, 1974) and the new-born lamb (Douglas Jones *et al.* 1975).

As in the lamb, no uptake of ketone bodies was demonstrated, in contrast to the uptake of ketones which has been shown to occur in rat brain in the first weeks of post-natal life (Hawkins, Williamson & Krebs, 1971), in new-born infants (Kraus *et al.* 1974), and in new-born dogs (Gregoire *et al.* 1978). This finding was not surprising as Hawkins *et al.* (1971) have shown that uptake of ketone bodies by brain is

dependant upon raised arterial concentrations which were consistently low in these animals.

Quantitative measurements of cerebral blood flow and metabolism in normal new-born animals *in vivo* only exist for the calf (present results), lamb (Purves & James, 1969) and dog (Hernandez *et al.* 1978; Gregoire *et al.* 1978); semi-quantitative measurements have been made in the rat (Barker, 1965; Moore, Lione, Regen, Tarpley & Raines, 1971). Average flow through grey matter in the lamb was 87 ml. $100\,g^{-1}.min^{-1}$, measured by intra-carotid arterial injection of ^{133}Xe with estimation of its clearance by an external detector. Oxygen consumption was 84 μmole. $100\,g^{-1}.min^{-1}$, but cerebral venous samples were taken from the sagittal sinus and precisely how far the tissue drained by the sagittal sinus corresponded to the area from which flow was determined is uncertain. In addition, the measurements were made under sodium pentobarbitone anaesthesia, which is well known to depress both flow and metabolism. In the new-born dog, under nitrous oxide anaesthesia (70 % N_2O, 30 % O_2) cerebral blood flow was 23 ml. $100\,g^{-1}.min^{-1}$ and oxygen consumption 58 μmole. $100\,g^{-1}.min^{-1}$, compared to average values of 56 ml. $100\,g^{-1}.min^{-1}$ and 125 μmole. $100\,g^{-1}.min^{-1}$ in adult dogs under similar experimental conditions (Hernandez *et al.* 1978; Brennan, Patterson & Kessler, 1971). Higher values however have been reported in new-born dogs under the same conditions by Gregoire *et al.* (1978). They found a mean cerebral blood flow of 48 ± 5 ml. $100\,g^{-1}.min^{-1}$, cerebral oxygen consumption 96 μmole. $100\,g^{-1}.min^{-1}$ and cerebral glucose uptake 20 ± 7 μmole. $100\,g^{-1}.min^{-1}$. Barker (1965) attempted to measure cerebral blood flow in new-born rats by collecting cerebral venous blood from the lacerated confluens sinorum and reported a value of 31 ml. $100\,g^{-1}.min^{-1}$. Moore *et al.* (1971) have reported values of 34 ml. $100\,g^{-1}.min^{-1}$ for cerebral blood flow in new-born rats, calculated from the rate of accumulation of ^3H_2O in the brain. These compare with a value of 104 ml. $100\,g^{-1}.min^{-1}$ in the adult rat calculated by the same method. They also estimated cerebral glucose utilisation in the new-born rat to be approximately 10 % that in the adult.

Oxygen consumption *in vitro* of incubated slices of calf brain cortex has been reported to be 72 μmole. $100\,g^{-1}.min^{-1}$ (Hertz & Clausen, 1963), compared to the value obtained *in vivo* in this series of 94 μmole. $100\,g^{-1}.min^{-1}$. Values for cerebral blood flow, glucose utilization and oxygen consumption in the calf therefore appear to be higher than those in the new-born rat, similar to those in the dog, but lower than those in the lamb. Meaningful comparisons between species however are difficult for two reasons. Firstly, there is evidence that neuronal packing density (and hence cerebral metabolic rate per unit weight) varies considerably with brain size (Tower 1954). Secondly, although there is evidence that the biosynthetic and functional changes which occur during maturation of the brain are accompanied by developmental changes in cerebral blood flow and metabolism (Kennedy, Grave, Jehle & Sokoloff, 1972; Himwich & Fazekas, 1941; Tyler & Van Harreveld, 1942), their duration and their relation to the timing of birth varies widely (Dobbing, 1974).

A reduction in cerebral glucose uptake during hypoglycaemia, with loss of the normal stoichiometric relationship to cerebral oxygen consumption has been found in all studies. In contrast, the absolute changes in cerebral glucose uptake, oxygen consumption and blood flow, and the level of blood glucose concentration at which

measurable alterations occurred, have varied widely. In adult humans, substantial reductions in cerebral glucose uptake have been reported at mean arterial glucose concentrations of 1·72 m-mole/l. (Eisenberg & Seltzer, 1962), 1·89 m-mole/l. (Della Porta *et al.* 1964), and 2·56 m-mole/l. (Gottstein & Held, 1967). In the calf, a significant reduction of cerebral glucose uptake was only observed at arterial plasma glucose concentrations below 1·0 m-mole/l. Similarly, cerebral glucose uptake has been found to fall only at blood glucose concentrations below 1·22 m-mole/l. in anaesthetized dogs (Himwich & Fazekas, 1937), and at capillary plasma glucose concentrations below 1·0 m-mole/l. in anaesthetized sheep (Pappenheimer & Setchell, 1973).

It is not clear to what extent these differences reflect age or species differences, or which of several possible mechanisms underlie them. It may be that differences in the kinetic constants governing glucose transport between plasma and the brain intracellular compartment allow greater net transport during hypoglycaemia, or simply that a lower cerebral metabolic rate, and hence a lower normal net glucose uptake, allows a greater margin of safety when plasma glucose concentration falls.

Cerebral oxygen consumption during insulin hypoglycaemia in man has been reported to increase (Eisenberg & Seltzer, 1962), decrease (Kety *et al.* 1948) and remain unchanged (Gottstein & Held, 1967). Pappenheimer & Setchell (1973) reported a decrease in sheep under barbiturate anaesthesia, and Norberg & Siesjö (1976) found no change in rats under nitrous oxide anaesthesia. This variability in experimental results is not surprising, as not only does hypoglycaemia precipitate primary changes in neuronal function, which range from coma to seizures and hence span the complete spectrum of metabolic demand but it also produces numerous peripheral neural and neuroendocrine responses (such as release of catecholamines from the adrenal medulla) which may alter the cerebral metabolic rate. In the present study cerebral oxygen consumption during severe hypoglycaemia was found to vary with the neurological state of the animals. The precise extent of the rise during seizures cannot be determined from these data however, as both cerebral blood flow and metabolism are likely to fluctuate when seizures occur. These results provide evidence that the changes in cerebral oxygen consumption associated with decreased consciousness and with generalized seizures resulting from hypoglycaemia do not differ qualitatively from those found in similar states arising from other causes.

The effect of hypoglycaemia on cerebral blood flow is controversial. In the classical study of Kety and his colleagues (Kety *et al.* 1948), cerebral blood flow was unchanged in four comatose patients, but increased in a fifth. Eisenberg & Seltzer (1962) did not find a significant increase in cerebral blood flow in their hypoglycaemic patients, and Pappenheimer & Setchell (1973) reported no increase in it in their study of anaesthetized sheep and rabbits. However, Della Porta *et al.* (1964) recorded an increase in cerebral blood flow in ten out of fourteen comatose patients, and Norberg & Siesjö (1976) showed that it increased two to threefold in severe hypoglycaemia in rats under nitrous oxide anaesthesia. In this study a significant change in cerebral blood flow during hypoglycaemia was only recorded during generalized seizures; the increase which occurred can be ascribed to the known coupling of cerebral blood flow to metabolic rate.

The fact that cerebral glucose uptake fell below that required to account for the cerebral oxygen consumption during profound hypoglycaemia shows that alternative substrates must have been oxidized under these conditions. Many metabolites which might be utilized during hypoglycaemia have been suggested, and evidence has been advanced for the oxidation of endogenous glycogen, amino acids, proteins, nucleic acids and phospholipids (Kerr & Ghantus, 1936; Abood & Geiger, 1955; Tews *et al.* 1965; Lewis *et al.* 1974; Dawson, 1950; Hinzen, Becker & Muller, 1970). In this study evidence was obtained for cerebral utilization of both circulating lactate and endogenous amino acids during hypoglycaemia.

Ammonia is known to accumulate in brain tissue deprived of an adequate supply of glucose. This has been demonstrated both *in vitro* (Weil-Malherbe, 1936; Weil-Malherbe & Gordon, 1971) and *in vivo* (Tews *et al.* 1965; Lewis *et al.* 1977) by the measurement of brain tissue ammonia concentrations. A net loss of ammonia by the brain to the circulation has been observed during insulin hypoglycaemia in the anaesthetized dog (Tews *et al.* 1965), but could not be quantified as cerebral blood flow was not measured. Lewis *et al.* (1974) noted that the net accumulation of free ammonia in the brains of their hypoglycaemic rats could almost account for the losses that corresponded to hydrolysis of glutamine and deamination of AMP, and suggested that relatively small amounts of ammonia were lost to the circulation. In these animals a significant though small net loss of ammonia from the brain occurred during hypoglycaemia and the rate of loss was higher in convulsing than in comatose animals. These results provide confirmatory evidence that a rise in cerebral ammonia concentration accompanies hypoglycaemia, and show that this occurs in the new-born animal. They also strongly suggest that endogenous amino acids were being utilized as alternative substrates (Lewis *et al.* 1974). Although ammonia production was significantly higher in convulsing animals in these experiments, a causative role for ammonia in the initiation of hypoglycaemia seizures cannot be inferred, as seizures themselves are associated with increased cerebral ammonia production (King, Carl & Lao, 1974).

Brain tissue can metabolize lactate *in vitro* (McIlwain, 1953), and the possibility that circulating lactate might be used as a substrate by the brain *in vivo* was first considered many years ago (McGinty, 1929; Wortis, Bowman & Goldfarb, 1941). Recent evidence suggests that a stereo-specific, saturable carrier system for lactate exists at the blood–brain barrier (Oldendorf, 1971; Nemoto, Hoff & Severinghaus, 1974) and cerebral uptake of lactate equivalent to about 30% of observed cerebral oxygen consumption has been reported in hypolycaemic, anaesthetized dogs when the blood lactate concentration was elevated by infusion of sodium lactate (Nemoto *et al.* 1974). Comline & Edwards (1968) observed the rise in blood lactate concentration which accompanies insulin hypoglycaemia in the calf during the first 24 hr after birth, and suggested that cerebral lactate utilization might be a factor in the resistance to hypoglycaemia of the new-born calf. In the present experiments, profound hypoglycaemia was associated with a marked rise in blood lactate concentration, together with uptake of lactate by the brain amounting to 6 μmole. $100\,g^{-1}.min^{-1}$ (sufficient if oxidized to account for 21% of the observed cerebral oxygen consumption); in older calves the blood lactate concentration rose to a lesser degree and there was no net uptake of lactate by the brain. The results of the

present experiments confirm the contention that lactate is taken up by the brain during insulin hypoglycaemia in the new-born calf, in sufficient amounts to meet a substantial proportion of the brain's energy requirement. The extent to which this may be responsible for the rarity with which hypoglycaemic convulsions occur at this age is uncertain, however, as the precise mechanisms underlying neurological dysfunction during hypoglycaemia are unknown and it has yet to be established that simple energy failure is a causative factor (Ferrendelli & Chang, 1973).

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REFERENCES

ABOOD, L. G. & GEIGER, A. (1955). Breakdown of proteins and lipids during glucose free perfusion of the cat's brain. *Am. J. Physiol.* **182**, 557–560.

AUKLAND, K., BOWER, B. F. & BERLINER, R. W. (1964). Measurement of local blood flow with hydrogen gas. *Circulation Res.* **14**, 164–187.

BARKER, J. N. (1965). Fetal and neonatal cerebral blood flow. *Am. J. Physiol.* **210**, 897–902.

BARKER, J. N. & BRITTON, H. G. (1957). The enzymatic determination of L(+) lactic acid. *J. Physiol.* **138**, 3–4*P*.

BERGMEYER, H. U. (1974). *Methods of Enzymatic Analysis*. 2nd ed. New York and London: Academic Press.

BRENNAN, R. W., PATTERSON, R. H. & KESSLER, J. (1971). Cerebral blood flow and metabolism during cardiopulmonary bypass: evidence of micro-embolic encephelopathy. *Neurology, Minneap.* **21**, 665–672.

COMLINE, R. S. & EDWARDS, A. V. (1968). The effects of insulin on the newborn calf. *J. Physiol.* **198**, 383–404.

DA FONSECA-WOLLHEIM, R. (1973). Direkt plasma ammoniakbessimmung ohne enteweissung. *Z. klin. Chem. klin. Biochem.* **11**, 426–431.

DAMESHEK, W. & MYERSON, A. (1935). Insulin hypoglycaemia: mechanism of the neurologic symptoms. *Archs. Neurol. Psychiat., Chicago* **33**, 1–18.

DAWSON, R. M. C. (1950). Studies on the glutamine and glutamic acid content of the rat brain during insulin hypoglycaemia. *Biochem. J.* **47**, 386–391.

DELLA PORTA, P., MAIOLO, A. T., NEGRI, V. U. & ROSSELLA, E. (1964). Cerebral blood flow and metabolism in therapeutic insulin coma. *Metabolism* **13**, 131–140.

DOBBING, J. (1974). The later development of the brain and its vulnerability. *Scientific Foundations of Paediatrics*, ed. DAVIS, J. A. & DOBBING, J., pp. 565–577. London: William Heinemann.

DOUGLAS JONES, M., BURD, L. I., MAKOWSKI, E. L., MESCHIA, G. & BATTAGLIA, F. C. (1975). Cerebral metabolism in sheep: a comparative study of the adult, the lamb and the fetus. *Am. J. Physiol.* **229**, 235–239.

EDWARDS, A. V. (1964). Resistance to hypoglycaemia in the newborn calf. *J. Physiol.* **171**, 46–47*P*.

EISENBERG, S. & SELTZER, H. S. (1962). The cerebral metabolic aspects of acutely induced hypoglycaemia in human subjects. *Metabolism* **11**, 1162–1168.

FERRENDELLI, J. A. & CHANG, M. (1973). Brain metabolism during hypoglycaemia. *Archs Neurol., Chicago* **28**, 173–177.

FIESCHI, C., BOZZAO, L. & AGNOLI, A. (1965). Regional clearance of hydrogen as a measure of cerebral blood flow. *Acta. neurol. scand.* **14**, suppl. 41, 46–52.

GARDINER, R. M. (1978). A class preparation for the demonstration of factors affecting cerebral blood flow and oxidative metabolism. *J. Physiol.* **284**, 24–25.

GOTOH, F., MEYER, J. S. & TOMITA, M. (1966). Hydrogen method for determining cerebral blood flow in man. *Archs Neurol., Chicago.* **15**, 549–559.

GOTTSTEIN, U. & HELD, K. (1967). Insulinwirkung auf den menschlichen Hirnmetabolisms von Stoffwechselgesunden und Diabetikern. *Klin. Wschr.* **45**, 18–23.

GREGOIRE, N. M., GJEDDE, A., PLUM, F. & DUFFY, T. E. (1978). Cerebral blood flow and cerebral metabolic rates for oxygen, glucose and ketone bodies in newborn dogs. *J. Neurochem.* **30**, 63–69.

HAWKINS, R. A., WILLIAMSON, D. H. & KREBS, H. A. (1971). Ketone body utilisation by adult and suckling rat brain *in vivo*. *Biochem. J.* **122**, 13–18.

HERNANDEZ, M. J., BRENNAN, R. W., VANNUCCI, R. C. & BOWMAN, G. S. (1978). Cerebral blood flow and oxygen consumption in the newborn dog. *Am. J. Physiol.* **234**, *R*209–215.

HERTZ, L. & CLAUSEN, T. (1963). Effects of potassium and sodium on respiration: their specificity to slices from certain brain regions. *Biochem. J.* **89**, 526–533.

HIMWICH, H. E. & FAZEKAS, J. F. (1937). The effect of hypoglycaemia on the metabolism of the brain. *Endocrinology* **21**, 800–807.

HIMWICH, H. E. & FAZEKAS, J. F. (1941). Studies of the metabolism of the brain of infants and adult dogs. *Am. J. Physiol.* **132**, 454–459.

HINZEN, D. H., BECKER, P. & MULLER, U. (1970). Einfluss von insulin auf den regionalen phospholipidstoffwechsel des kanninchengehirns *in vivo*. *Pflügers Arch.* **321**, 1–14.

KENNEDY, C., GRAVE, G. D., JEHLE, J. W. & SOKOLOFF, L. (1972). Changes in blood flow in the component structures of the dog brain during postnatal maturation. *J. Neurochem.* **19**, 2423–2433.

KERR, S. E. & GHANTUS, M. (1936). The carbohydrate metabolism of the brain. II. The effects of varying the carbohydrate and insulin supply on the glycogen free sugar, and lactic acid in mammalian brain. *J. biol. Chem.* **116**, 9–20.

KETY, S. S. & SCHMIDT, C. F. (1945). The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *Am. J. Physiol.* **143**, 53–66.

KETY, S. S., WOODFORD, R. B., HARMEL, M. H., FREYHAN, F. A., APPEL, K. E. & SCHMIDT, C. F. (1948). Cerebral blood flow and metabolism in schizophrenia. The effects of barbiturate semi-narcosis, insulin coma and electroshock. *Am. J. Psychiat.* **104**, 765.

KING, L. J., CARL, J. L. & LAO, L. (1974). Brain amino acids during convulsions. *J. Neurochem.* **22**, 307.

KRAUS, H., SCHLENKER, S. & SCHWEDESKY, D. (1974). Developmental changes of cerebral ketone body utilisation in human infants. *Hoppe-Seyler's Z. physiol. Chem.* **355**, S, 164–170.

LEWIS, L. D., LJUNGGREN, B., NORBERG, K. & SIESJÖ, B. K. (1974). Changes in carbohydrate substrates, amino acids and ammonia in the brain during insulin-induced hypoglycaemia. *J. Neurochem.* **23**, 659–671.

LINDEN, R. J., LEDSOME, J. R. & NORMAN, J. (1965). Simple methods for the determination of carbon dioxide and oxygen in blood. *Br. J. Anaesth.* **37**, 77–88.

MCHENRY, L. C., JUN. (1963). Determination of cerebral blood flow by a krypton-85 desaturation method. *Nature, Lond.* **200**, 1297.

MCGINTY, D. A. (1929). The regulation of respiration; variations in the lactic metabolism in the intact brain. *Am. J. Physiol.* **88**, 312–325.

MCILWAIN, H. (1953). Substances which support respiration and metabolic response to electrical impulses in human cerebral tissues. *J. Neurol. Neurosurg. Psychiat.* **16**, 257–266.

MOORE, T. J., LIONE, A. P., REGEN, D. M., TARPLEY, H. L. & RAINES, P. L. (1971). Brain glucose metabolism in the newborn rat. *Am. J. Physiol.* **221**, 1746–1753.

NEMOTO, E. M., HOFF, J. T. & SEVERINGHAUS, J. W. (1974). Lactate uptake and metabolism by brain during hyperlactatemia and hypoglycaemia. *Stroke* **5**, 48–53.

NILSSON, B. & SIESJÖ, B. K. (1976). A method for determining blood flow and oxygen consumption in the rat brain. *Acta. physiol. scand.* **96**, 72–82.

NORBERG, K. & SIESJÖ, B. K. (1976). Oxidative metabolism of the cerebral cortex of the rat in insulin-induced hypoglycaemia. *J. Neurochem.* **26**, 345–352.

OLDENDORF, W. H. (1971). Blood brain barrier permeability to lactate. *Eur. Neurol.* **6**, 49–55.

PAPPENHEIMER, J. R. & SETCHELL, B. P. (1973). Cerebral glucose transport and oxygen consumption in sheep and rabbits. *J. Physiol.* **233**, 529–551.

PURVES, M. J. & JAMES, I. M. (1969). Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb. *Circulation Res.* **25**, 651–667.

SHELLEY, H. J. & NELIGAN, G. A. (1966). Neonatal hypoglycaemia. *Br. med. Bull.* **22**, 34–39.

TEWS, J. K., CARTER, S. H. & STONE, W. E. (1965). Chemical changes in the brain during insulin hypoglycaemia and recovery. *J. Neurochem.* **12**, 679–693.

TOWER, D. B. (1954). Structure and functional organization of mammalian cerebral cortex: the correlation of neurone density with brain size. *J. comp. Neurol.* **101**, 19–52.

TYLER, D. B. & VAN HARREVELD, A. (1942). The respiration of the developing brain. *Am. J. Physiol.* **136**, 600–603.

WEIL-MALHERBE, H. (1936). Studies on brain metabolism. 1. The metabolism of glutamic acid in brain. *Biochem. J.* **30**, 665.

WEIL-MALHERBE, H. & GORDON, J. (1971). Amino-acid metabolism and ammonia formation in brain slices. *J. Neurochem.* **18**, 1659–1672.

WORTIS, J., BOWMAN, K. M. & GOLDFARB, W. (1941). Availability of lactic acid for brain oxidations. *J. Neurophysiol.* **4**, 243–249.